THE PROVENTRICULAR FLANGES IN MOSQUITO LARVAE[1] [2]

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ABSTRACT: Examination of stained thin sections by transmission electron microscopy showed that mature larvae of species in 9 different genera of mosquitoes have 1, 2, or more circumferential flanges around the proventriculus. Differences could supply taxonomically useful characters. Similar flanges have been recorded for some chironomid larvae, and flanges with serrated edges in black fly larvae.

DESCRIPTORS: Mosquito larva, *Simulium* larva, proventriculus, cuticle.

The proventriculus of late instar larvae of *Aedes triseriatus* and *Aedes aegypti* are surrounded by two circumferential flanges as described in detail by Romoser and Venard (1967) and Richards and Richards (1971, 1976). This structure is illustrated in cut-away surface view in Figure 1 and in longitudinal section in Figure 2. Since the situation described for larvae of *Anopheles plumbeus* by Wigglesworth (1930) seemed somewhat different, a survey of available mosquito larvae was made using thin sections and transmission electron microscopy.

The larvae were fixed in cold buffered glutaraldehyde followed by O_sO_4, embedded in an epoxy resin, sectioned, stained with uranyl acetate and lead citrate, and examined in a Philips 300 EM.

Fourth instar larvae of culicine mosquitoes fall into two groups: those which like *Aedes* have two flanges on a swollen ring (Figs. 1-6), and those which have only one flange (Figs. 7-8). It is to be noted that one of the species of *Culiseta* has two flanges (Fig. 3) whereas the other has only one (Fig. 7), but these two species are in different subgenera. Two genera are not illustrated: *Coquillettidia ("Mansonia") perturbans* is very close to Figure 7, and *Orthopodomyia signifera* is also similar to Figure 7 but flat as Figure 8 is.

The single specimen of *Uranotaenia sapphirina* showed no swollen ring (Fig. 6). This is probably the true condition since the underlying epidermis seems to be in a quiescent rather than a

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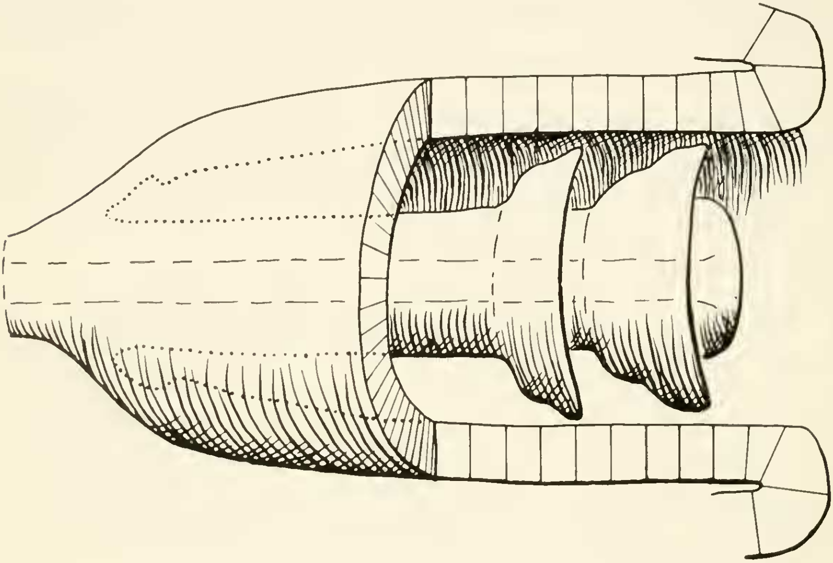


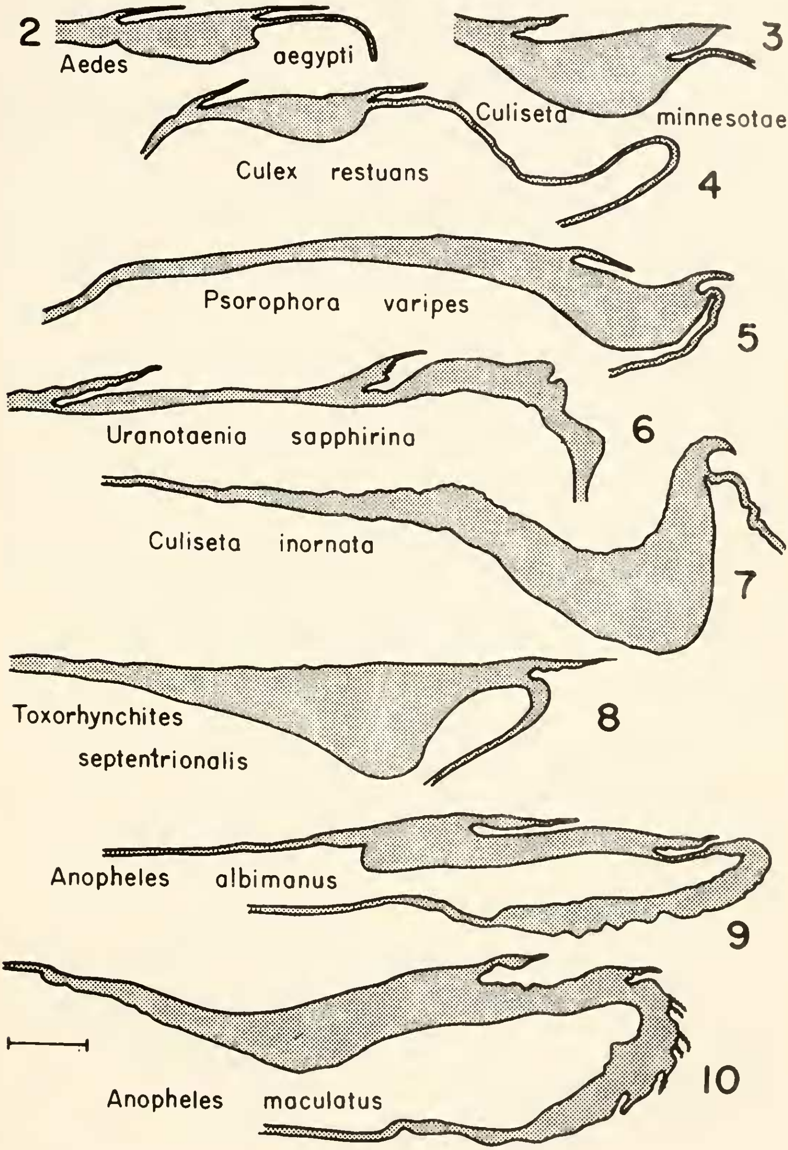
Fig. 1. Diagrammatic hemisectional view of the anterior end of the midgut of a fourth instar larva of *Aedes aegypti* with the flanged proventriculus protruding into it. Head to left. (After Richards & Richards, 1976).

secretory phase. But it is not certain that this larva, although large, was in the fourth instar.

The situation in larvae of anopheline mosquitoes is more variable and in some species more complex.

Within the genus *Anopheles* there are various subdivisions. A half dozen subgenera are recognized, the three largest being *Nyssorhynchus, Cellia* and *Anopheles.* Of the species treated, *A. albimanus* (Caribbean region) belongs to the subgenus *Nyssorhynchus, A. maculatus* (Malaya) and *A. balabacensis* (Philippines) to *Cellia,* and *A. freeborni* (western U.S.A.), *A. quadrimaculatus* (eastern U.S.A.) and *A. plumbeus* (Europe) to the subgenus *Anopheles.*

Fourth instar larvae of all of the species of *Anopheles* differ from culicines in having not only a swollen ring at the level of the flanges but also a swollen cuticle extending on around the end of the proventriculus and a corresponding distance up the esophagus (compare Figs. 9 & 10 with 4 & 5). But there are differences in the flanges: in *A. albimanus* (Fig. 9) and *A. balabacensis* there are two circumferential flanges equivalent to those seen in many culicines; in *A. maculatus* (Fig. 10) there are two major circumferential

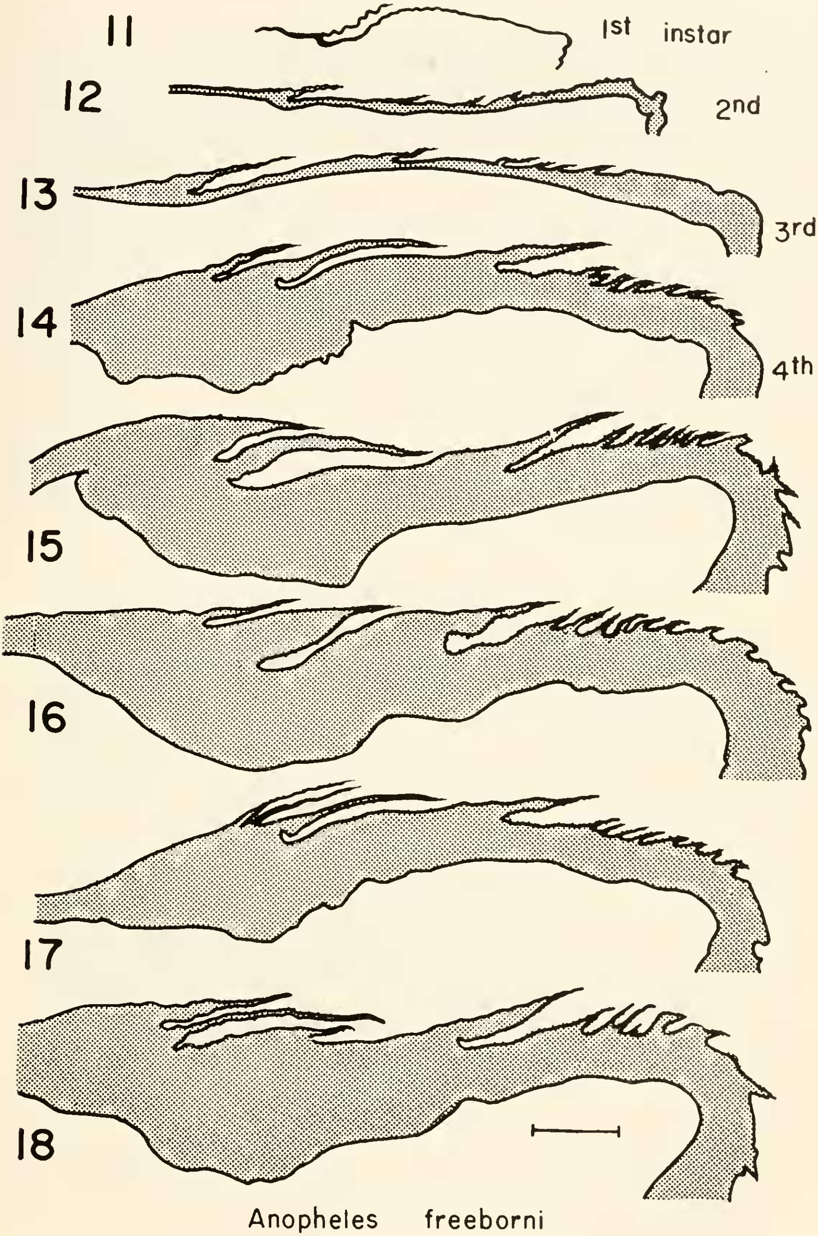


Figs. 2-10. Tracings of the flanges and cuticular thickenings as seen in longitudinal sections of the proventriculus of fourth instar larvae of the indicated mosquitoes. All at the same magnification (bar = 10 μm) except Fig. 8 which is at 1/3 this magnification.

flanges plus a half dozen minor wrinkles at the tip of the proventriculus; but in *A. freeborni* (Figs. 14-18) and *A. quadrimaculatus* (presumably also *A. plumbeus*) there are 3 or 4 major circumferential flanges plus up to a dozen minor ones in the area between the major flanges and the tip of the proventriculus. Serial sections show that the major flanges mostly extend all the way around the proventriculus although sometimes one does not, but the minor flanges commonly extend only part of the way. For documentation of this statement, we examined 21 successive longitudinal sections from one larva of *A. freeborni*. These sections averaged 0.08 μm thick and therefore represent a distance of more than 1.5 μm. The first and twenty-first sections (and all intervening ones) were essentially the same (Figs. 15-16). From another larva we examined every twentieth section for a distance of 320 sections (ca 25 μm). All of these showed 4 major flanges, seemingly continuous but of fluctuating size, plus 6-8 smaller ones (Figs. 17-18). Clearly one is dealing with flanges rather than spicules (Fig. 19).

For most of the species a single larva or only a few larvae were examined. But for *Anopheles freeborni* we examined sections from 5 fourth instar larvae, for *A. quadrimaculatus* from 4, and for *Aedes aegypti* from hundreds of larvae during various studies. In *A. aegypti* there is only slight variation from section to section and from specimen to specimen (Richards and Richards, 1976). Their general similarity to *A. aegypti* suggests that the same will be true for most culicines. But in the two species of *Anopheles* there is both considerable individual variation (Figs. 14 vs 15-16 vs 17-18) and a smaller amount of circumferential variation.

The development of flanges has been worked out in detail for *Aedes aegypti* (Richards and Richards, 1976). Rings of several dozen cells cooperate to produce circumferential cytoplasmic flanges which, when the new cuticle is secreted, become solid cuticular flanges. Sections from single pharate individuals of *Anopheles albimanus, Culiseta minnesotae* and the chironomid *Psectrocladius* sp. indicate that this is the general method for flange formation. But the fully-formed condition can be different in different instars. In *A. aegypti* the first instar and most of the second instar larvae have no flanges, but most third instar larvae have 2 flanges and the fourth instar ones always have 2 flanges on a swollen ring. In *Anopheles albimanus* the first and second instars



Figs. 11-18. Tracings of the flanges and cuticular thickenings as seen in longitudinal sections of the proventriculus of larvae of *Anopheles freeborni.* We examined sections from 2 first instar, 2 second instar, 3 third instar, and 5 fourth instar larvae. Fig. 14 is from one specimen (= Fig. 19); Figs. 15-16 are from a set of serial sections from another specimen and are ca 1.5 μm apart; and Figs. 17-18 are from another set of serial sections and are ca 15 μm apart. All figures at the same magnification (bar = 10 μm).

each have a single delicate flange, the third instar has 2 flanges, and the fourth instar has 2 flanges on a swollen ring. In *Anopheles freeborni* the first instar has a single delicate flange (Fig. 11), the second instar has 3 or 4 flanges but there is no distinction between major and minor ones (Fig. 12), the third instar has 2 or 3 major and 4 or 3 minor ones (Fig. 13), and the fourth instar has 3 or 4 major flanges plus 6-10 minor ones on a considerably swollen cuticle (Figs. 14-18).

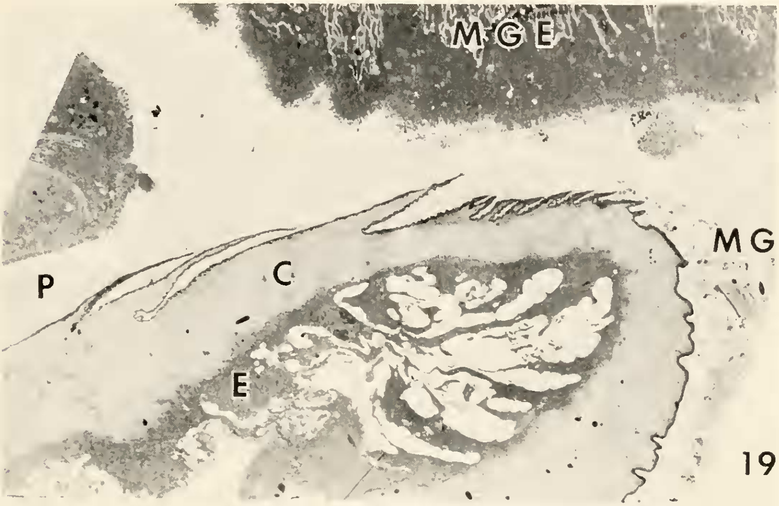
Sections of last instar larvae of a *Simulium* sp. (probably *venustum*) showed what at first looked like minute spicules as described by Puri (1925). Closer inspection, especially of cross sections of the proventriculus, revealed that these are really incomplete circumferential flanges with serrated tips (Fig. 20). Actually, this is little different from the old drawing by Puri except that he saw only the serrated tips without being able to resolve clearly the overlapping flanges.

Discussion

Clearly, circumferential flanges of cuticle around the larval proventriculus are to be found in a number of families of nematocerous Diptera, as first recorded by Vignon (1901). The larger ones of these seem to be continuous around the entire circumference; smaller ones may be continuous or extend only part way around. When such incomplete smaller flanges are present there are numerous ones with the result that flanges extend all the way around the proventriculus even though no single one does.

In mosquito larvae the flanges are formed around cytoplasmic projections which form a nearly uniform albeit somewhat ruffled collar (Fig. 1. Richards and Richards, 1976). In a black fly larva the cytoplasmic projections must have a serrated margin because they produce flanges with serrated edges (Puri, 1925, and Fig. 20). This is consistent with the fact that at the asterisk in Figure 20 there are cross sections of 4 cytoplasmic fingers with spacings similar to that of the serrated edge of the flange [the presence of microvilli at the cell-cuticle interface indicates that this specimen is still secreting cuticle].

In a mycetophilid larva Holmgren (1907) reported "minute spicules" on the cuticle of the proventriculus. Re-examination by electron microscopy is needed to see if they, like the spicules of



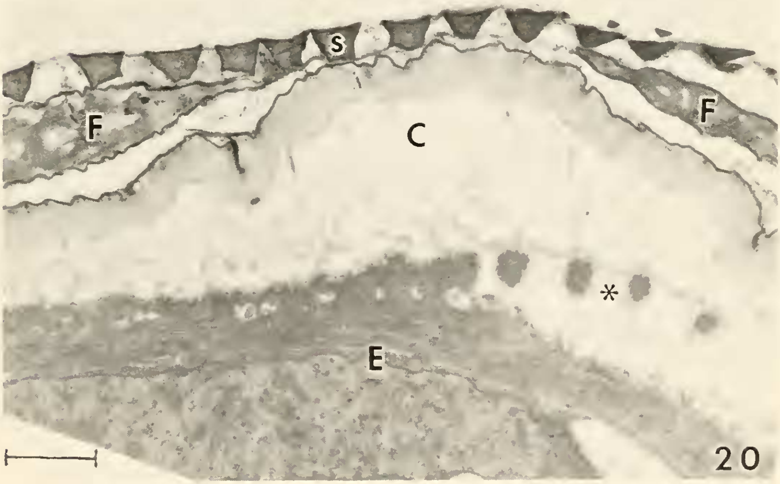


Fig. 19. Actual transmission electron microscopy picture of a longitudinal section of the proventriculus of a fourth instar larva of *Anopheles freeborni* (Fig. 14 was traced from this picture). The peritrophic membrane which lies against the flanges has been masked with "liquid paper" to permit the flanges to be seen clearly. Same magnification as Fig. 20.

Fig. 20. Cross section of proventriculus of a larva of *Simulium* sp. (probably *venustum*) showing overlapping flanges with serrated tips. A ring of serrated tips (S) are outside the more basal flanges (F) which are in turn separate from the general cuticle (C). Bar = 10 μm.

Abbreviations: C = cuticle of proventriculus; E = epidermis of proventriculus; F = circumferential cuticular flanges; MG = lumen of midgut; MGE = epithelium of midgut; P = pouch formed by proventriculus protruding into midgut; S = spicule of serrated tip of a flange.

Puri (1925) and Wigglesworth (1930), are to be re-interpreted.

It has been suggested that these flanges may function either in helping propel the peritrophic membrane posteriorly or in keeping food and bacteria out of the pouch where the PM is formed or both (Richards and Richards, 1971, 1976). Figure 21 is consistent with the second suggestion; a fortuitous dilatation at the level of the proventriculus shows the numerous bacteria and food particles in the lumen of the gut extending up to the first flange, and a few getting beyond the first flange but not beyond the second flange. Whatever the function, the possible use of these details as taxonomic characters is obvious. But, unfortunately, despite the size of these flanges it is difficult to work with them by light microscopy because the flanges and peritrophic membrane are usually squeezed together between proventriculus and midgut epithelium. Only occasional specimens have the parts so separated that the details can be seen in sections prepared for light microscopy. Whole mounts would show little when examined by light microscopy, and would show only half the features if examined by scanning electron microscopy. Thin sections prepared for transmission electron microscopy are really needed – and these require specially processed material and much work.

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REFERENCES

Holmgren, N. 1907. Monographische Bearbeitung einer schalentragenden Mycetophilidenlarve (*Mycetophila ancyliformans* n. sp.). Z wiss. Zool. 88: 1-77

Puri, I.M. 1925. On the life history and structure of the early stages of Simuliidae. Parasitology 17: 295-369.

Richards, A.G. and P.A. Richards. 1971. The origin and composition of the peritrophic membrane of the mosquito *Aedes aegypti*. J. Insect Physiol. 17: 2253-2275.

Richards, P.A. and A.G. Richards. 1976. Development and variation in flanges on the proventriculus of larvae of *Aedes aegypti*. J. Med. Entomol. (in press).

Romoser, W.S. and C.E. Venard. 1967. Development of the dorsal oesophageal diverticula in *Aedes triseriatus*. Ann. Entomol. Soc. Amer. 60: 617-623.

Vignon, P. 1901. Recherches de cytologie generale sur les epitheliums. Arch. Zool. exp. gen. 9: 371-715 (see pp. 382-396).

Wigglesworth, V.B. 1930. The formation of the peritrophic membrane in insects, with special reference to the larvae of mosquitoes. Q.J. Microsc. Sci. 73: 593-616.

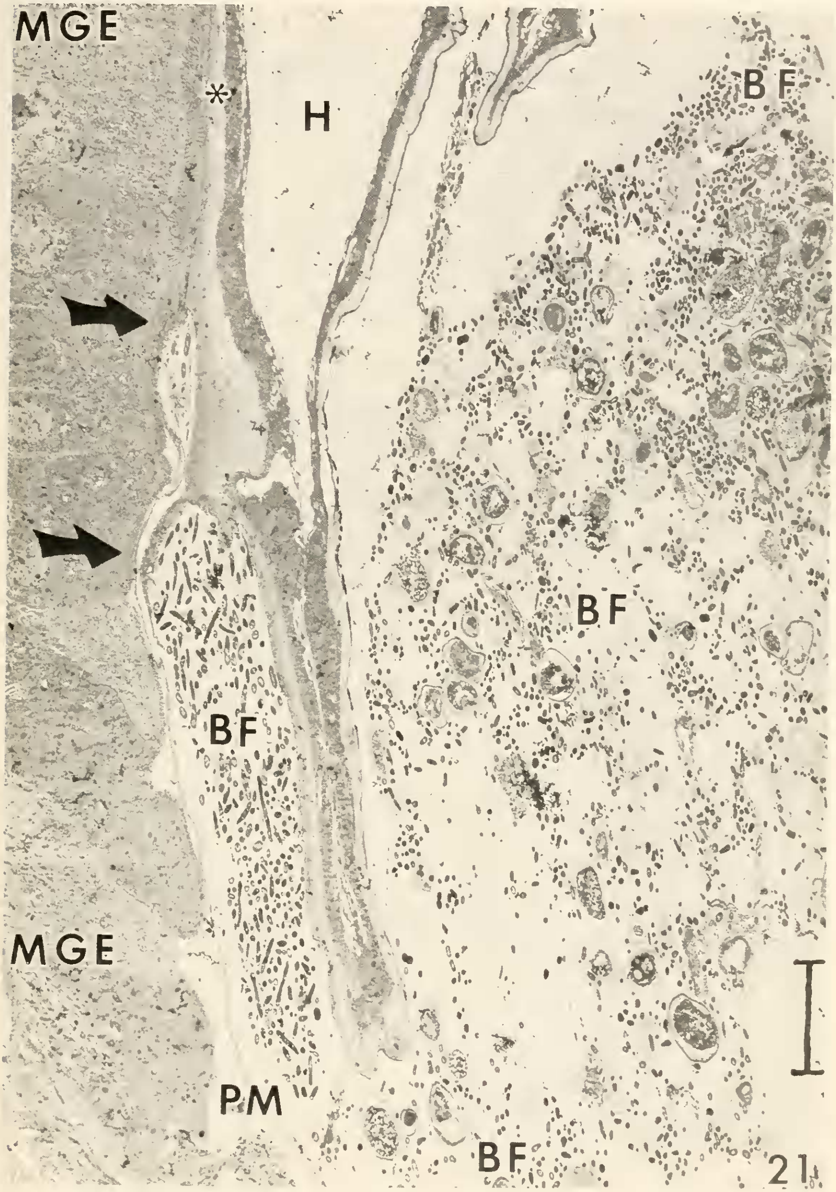

Fig. 21. Longitudinal section of a fourth instar larva of *Aedes aegypti* showing part of one side of the proventricular region. Head end of larva beyond top of picture. A dilatation shows that bacteria and food particles (BF) are mostly blocked from the pouch where peritrophic membrane is formed (*) by the first flange (lower arrow) and entirely by the second flange (upper arrow). Bar = 10 μm.

H = hemocoel within proventriculus; MGE = midgut epithelium; PM = fully formed peritrophic membrane.